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# ATCC

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## BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSES OF PATENT PROCEDURE

### INTERNATIONAL FORM

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT ISSUED PURSUANT TO RULE 7.3  
AND VIABILITY STATEMENT ISSUED PURSUANT TO RULE 10.2

To: (Name and Address of Depositor or Attorney)

Bristol-Myers Squibb Co.  
Attn: Robert J. Peach  
P.O. Box 4000  
Princeton, NJ 08543-4000

Deposited on Behalf of: Bristol-Myers Squibb Company

Identification Reference by Depositor:

Patent Deposit Designation

Plasmid L104EA29Ylg

PTA-2104

The deposit was accompanied by:    a scientific description    a proposed taxonomic description indicated above.

The deposit was received June 20, 2000 by this International Depository Authority and has been accepted.

AT YOUR REQUEST:   X   We will inform you of requests for the strain for 30 years.

The strain will be made available if a patent office signatory to the Budapest Treaty certifies one's right to receive, or if a U.S. Patent is issued citing the strain, and ATCC is instructed by the United States Patent & Trademark Office or the depositor to release said strain.

If the culture should die or be destroyed during the effective term of the deposit, it shall be your responsibility to    replace it with living culture of the same.

The strain will be maintained for a period of at least 30 years from date of deposit, or five years after the most recent request for a sample, whichever is longer. The United States and many other countries are signatory to the Budapest Treaty.

The viability of the culture cited above was tested June 28, 2000. On that date, the culture was viable.

International Depository Authority: American Type Culture Collection, Manassas, VA 20110-2209 USA.

Signature of person having authority to represent ATCC:

  
Barbara E. Coupé, Administrator, Patent Depository

Date: June 30, 2000

cc: Audrey F. Sher (Ref. Docket D0028)

## LEA29Y

- Fusion protein of mutated CTLA4-Ig and human IgG1 Fc domain
- Mutations at position 29 and 106 confer
  - Comparable binding to B7-1
  - 10-fold higher avidity to B7-2 than parent CTLA4-Ig molecule

# LEA29Y structure

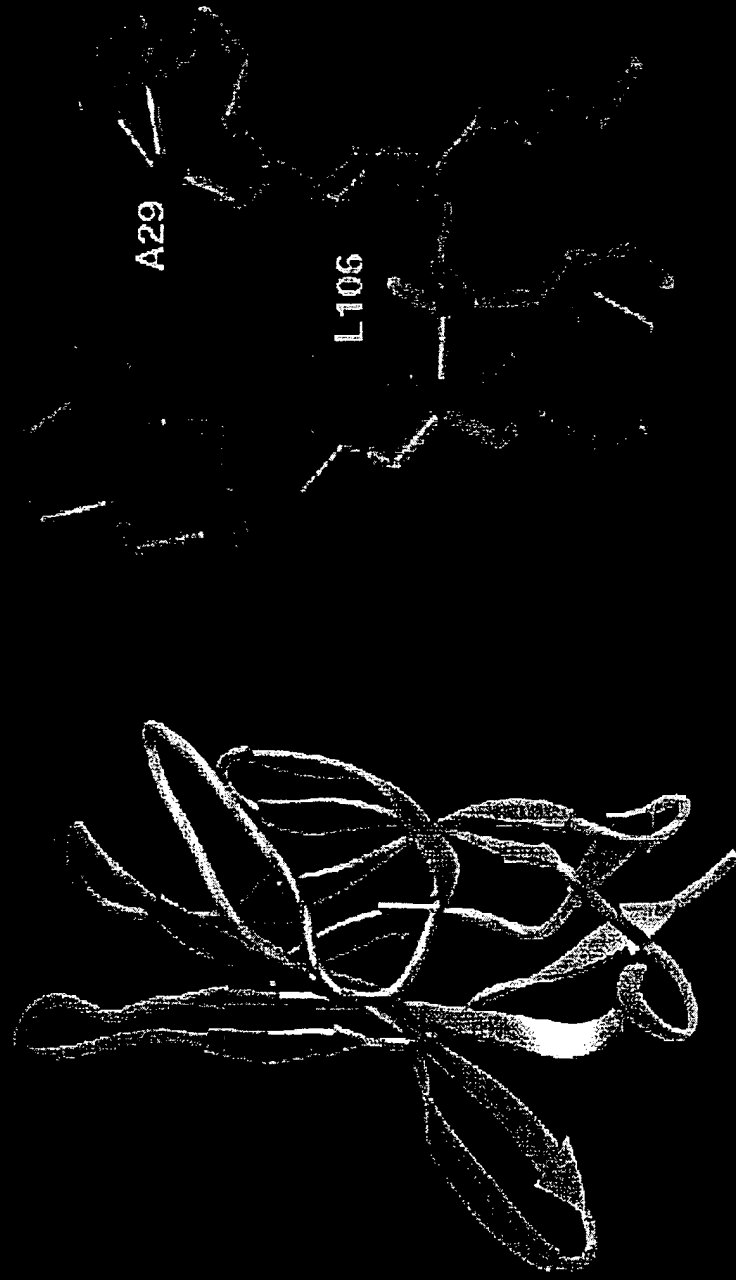
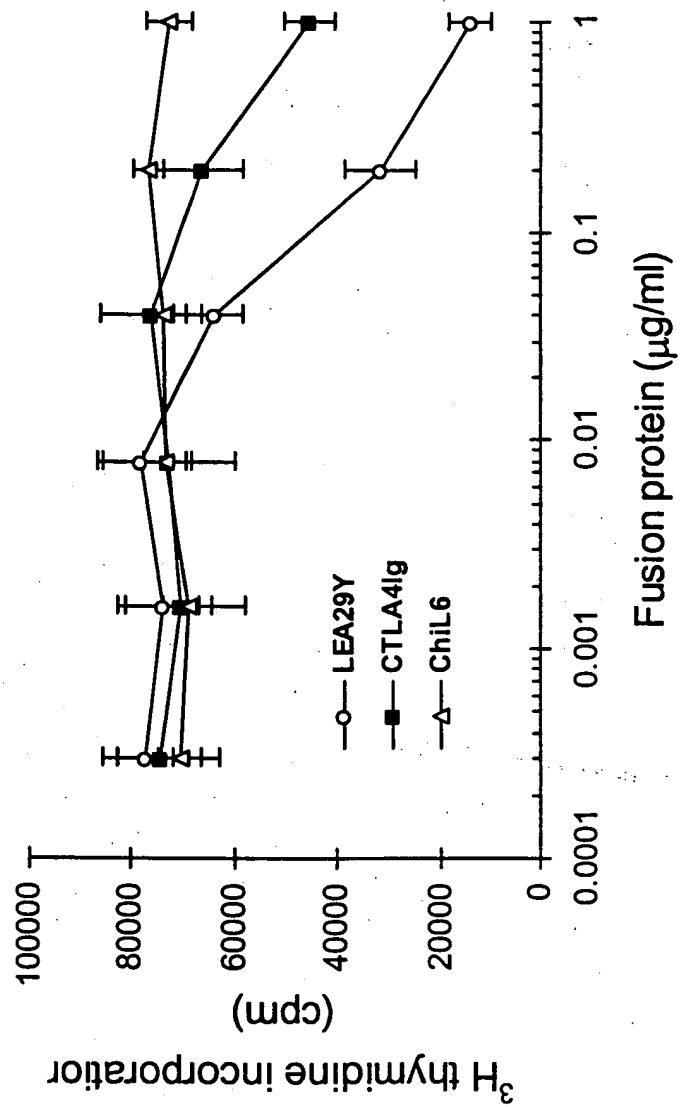


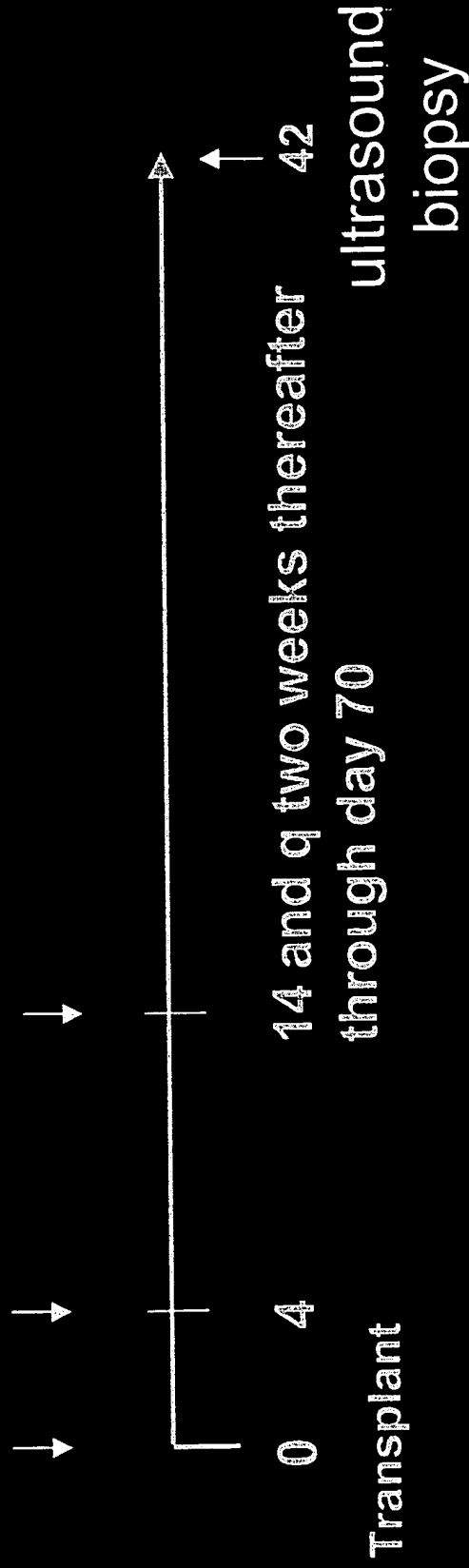
Figure 613. (left) A ribbon diagram of the CTLA-4 is shown with the CDR1- and CDR3-analogous loops colored green and magenta, respectively. (right) An expansion of the CDR1- and CDR3-analogous loops indicating the location of the avidity-enhancing mutations, L106 and A129.

# **A** Primary alloresponse



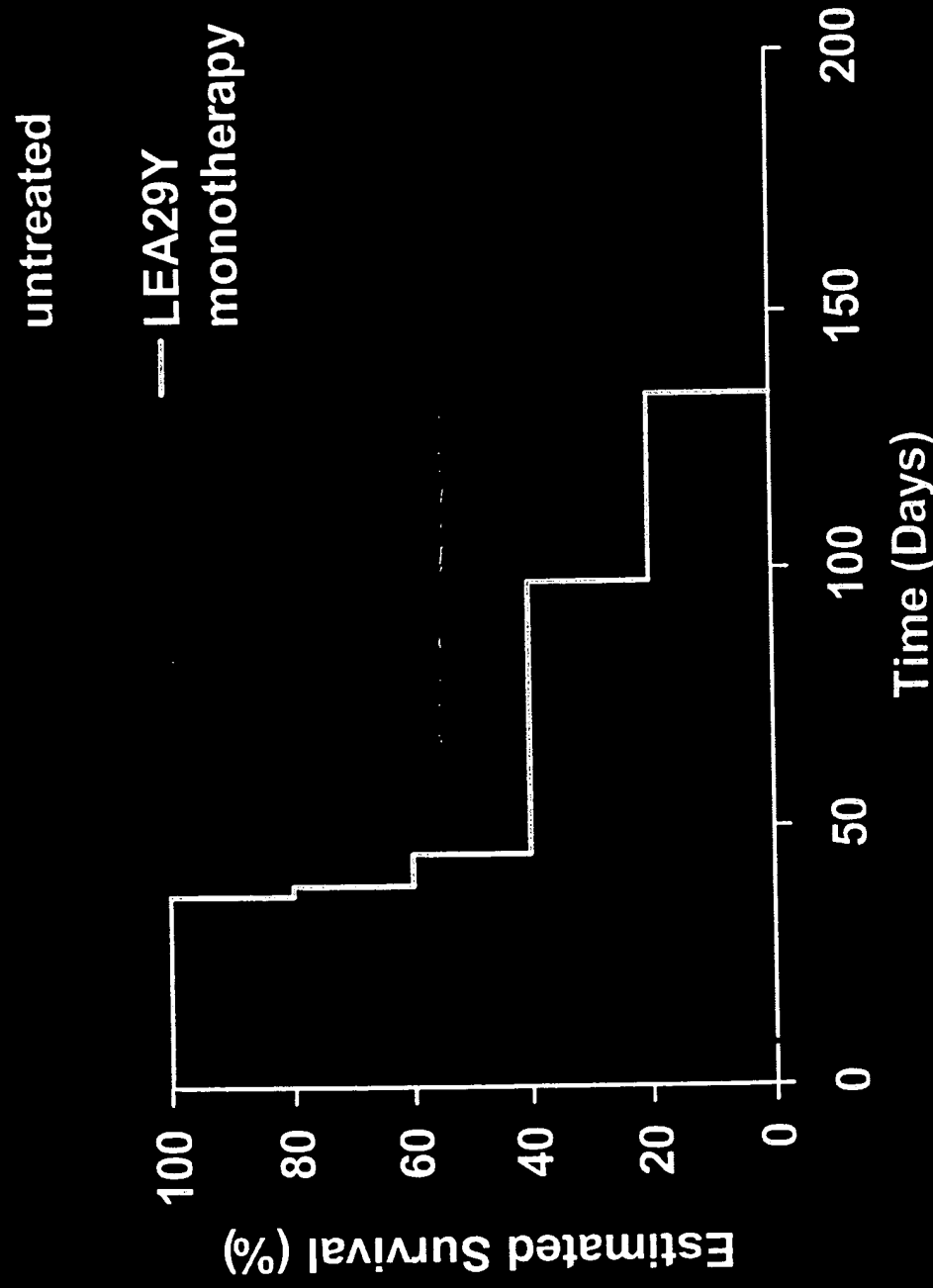
# Effect of LEA29Y monotherapy on Renal allograft survival in macaques

LEA29Y 10 15 20 mg/kg (trough serum conc > 30 ug/ml)



(no treatment of acute rejection episodes)

# LEA29Y monotherapy prolongs allograft survival in rhesus macaques



# LEA29Y MMF protocol

(20mg/day)

(2mg/day)

(1mg/day)

Solumedrol

15mg/kg bid

(15mg/kg qd

MMF

LEA29Y

10

15

20

↓

↓

↓

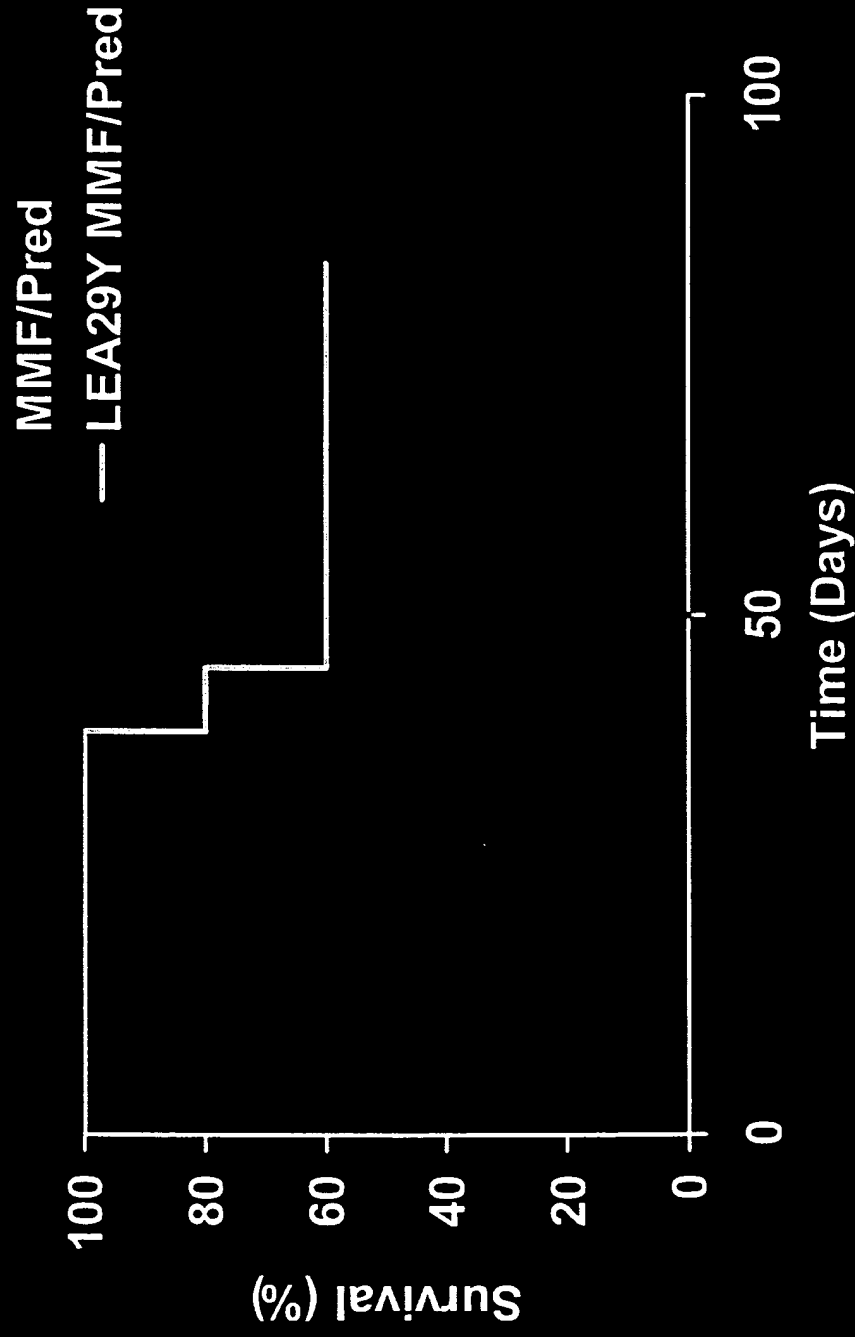
0

4

14 and q two weeks thereafter through day 70



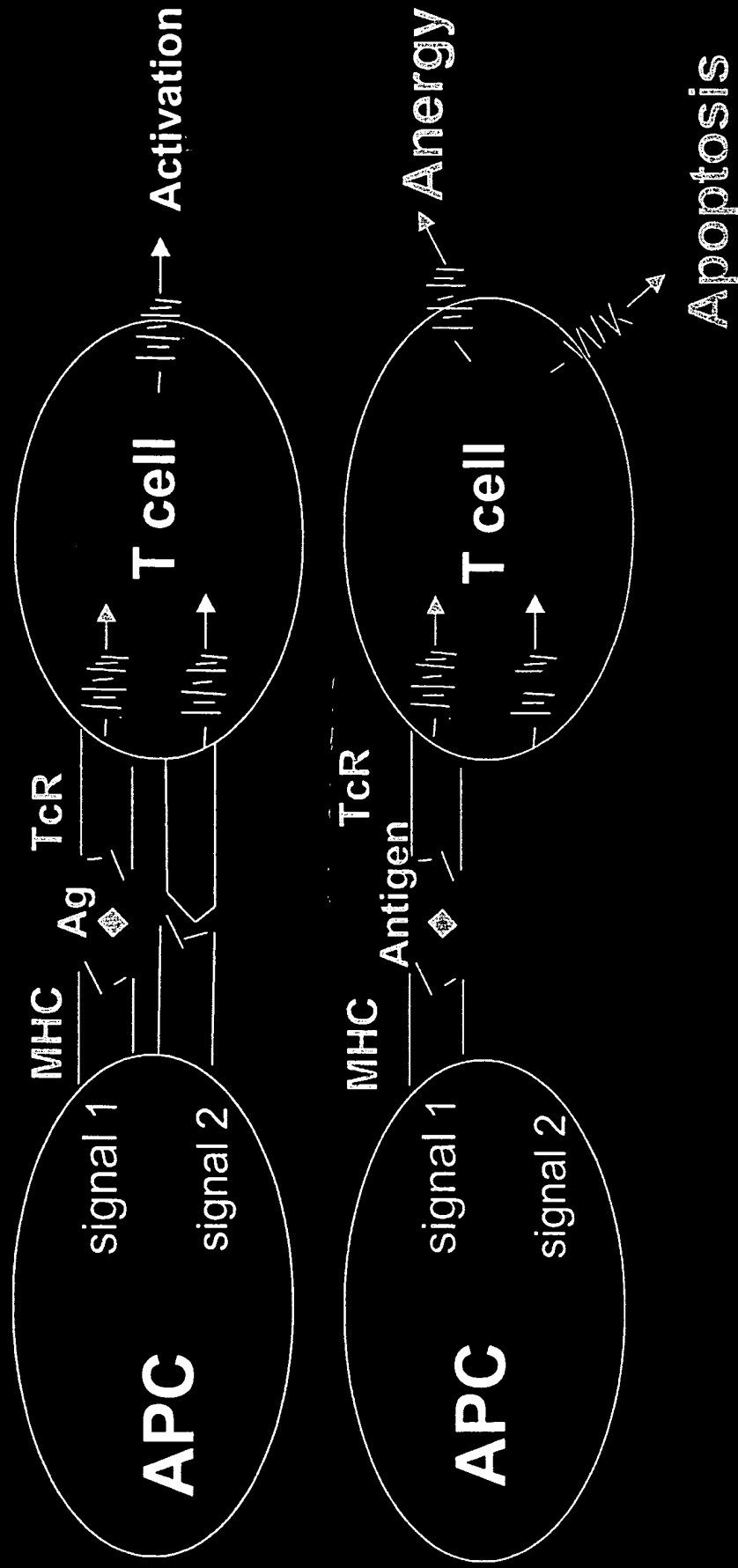
# Effects of LEA29Y, MMF and prednisone on renal allograft survival in rhesus macaques



# CD28 Summary

- Manipulation of the CD28 pathway inhibits allograft rejection and inhibits anti-donor Abs
- Second generation molecules (LEA29Y) may offer enhanced efficacy
- Addition of adjunctive agents will be necessary
  - Interactions with conventional immunosuppressants remains an unresolved issue
  - however addition of MMF/pred enhanced the efficacy of LEA29Y

# Two signal model of T cell activation



APC

B7-1 Inducible  
Low Abundance  
promotes Th1

B7-2 Inducible  
High Abundance  
promotes Th2

T Cell

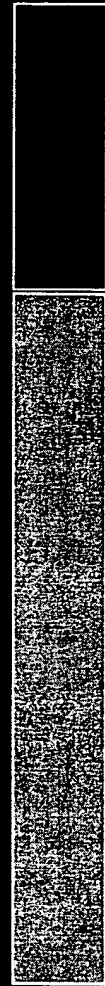
CD28 → kinases

- Constitutive
- High Abundance
- Low Avidity

CTLA4 → phosphatases

- Inducible
- Low Abundance
- High Avidity

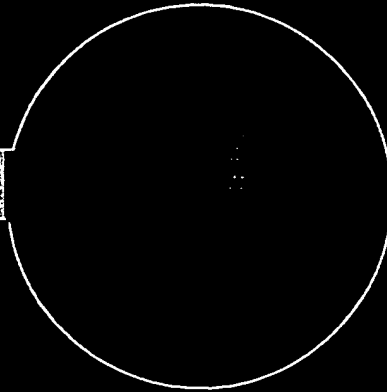
# CTLA4-Ig



CTLA4

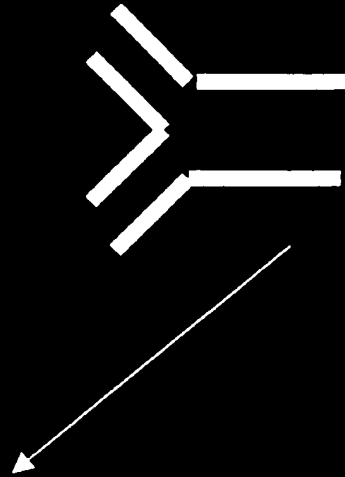
human Ig

CTLA4



High Avidity  
B7-1 > B7-2

- mouse
- rat
- rhesus
- human



Ig

# CD28 blockade: Lessons from murine models

- Agents
  - CTLA4-Ig- a potent inhibitor of T cell responses *in vitro* and *in vivo*
    - » Lenschow, et al. *Science* 1992
    - » Turka, et al. *PNAS* 1992
  - Anti B7 monoclonal antibodies- blockade of B7-1 and B7-2 required
    - » Lenschow et al 1995. *Transplantation* 60:1171.
    - » Pearson et al 1997. *Transplantation* 63:1463.
- Timing of blockade may influence efficacy/outcome
  - » Lin et al, *J Exp Med* 1993
  - » Sayegh et al, *J Exp Med* 1995
- Adjuncts enhance efficacy
  - DST
  - Anti-CD40L
  - Anti-CD4

# CD28 blockade: Lessons from murine models

- Mechanism of action
  - Cellular effects
    - » inhibition of T cell activation/expansion
  - » Anergy
  - » Immune deviation
  - » Peripheral deletion
  - Molecular mechanisms
    - » Preferential CTLA4 signaling
    - » altered balance of regulators of apoptosis
- Interactions with “conventional” immunosuppressive agents may be important
  - Calcineurin inhibitors and corticosteroids
  - Rapamycin

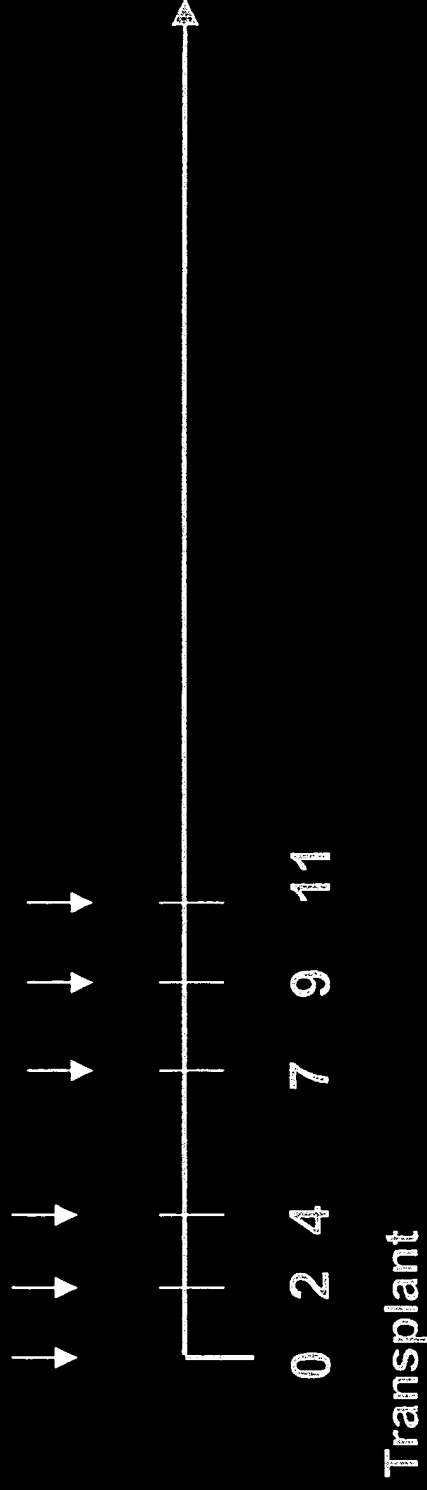
## CD28 blockade in primates

- CTLA4-Ig monotherapy has very modest effects on allograft survival and inhibits anti-donor antibody responses
  - Kirk, A.D et al 1997. PNAS 94,16:8789.
  - Levisetti et al 1997. JI 159:5187.
- Effect of anti-B71 and anti-B72 similar to CTLA4-Ig
  - Ossevoort et al 1999 Transplantation 68, 1010

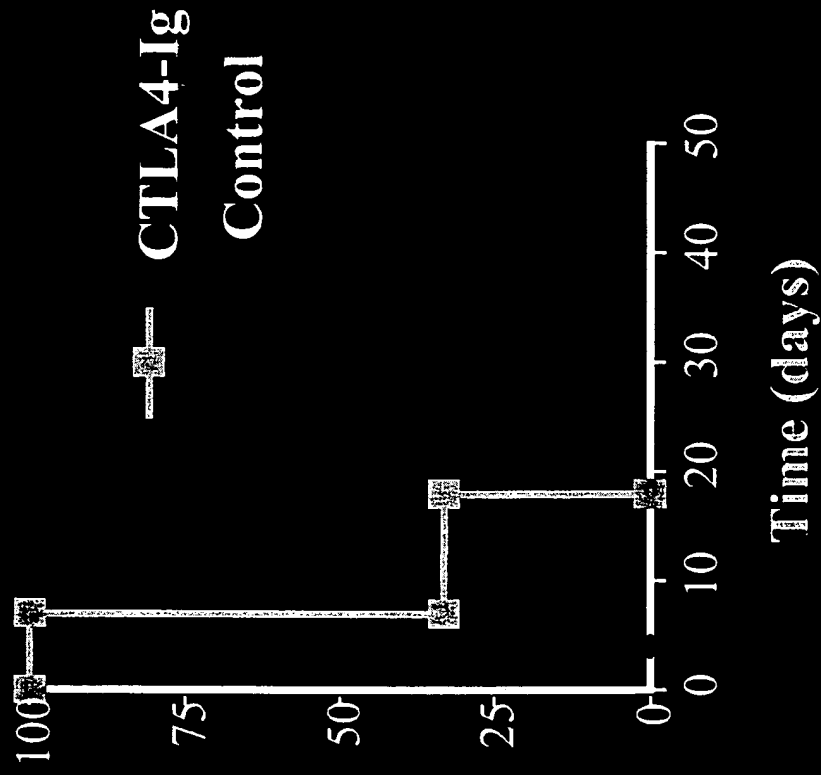
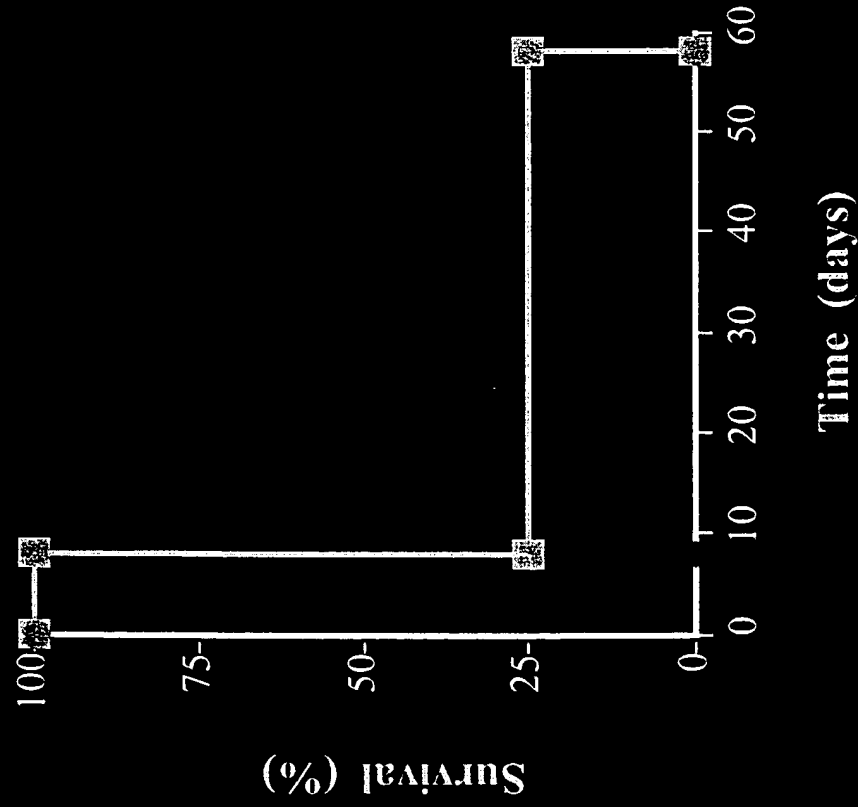


# Effect of CTLA4-Ig monotherapy on Renal allograft survival in macaques

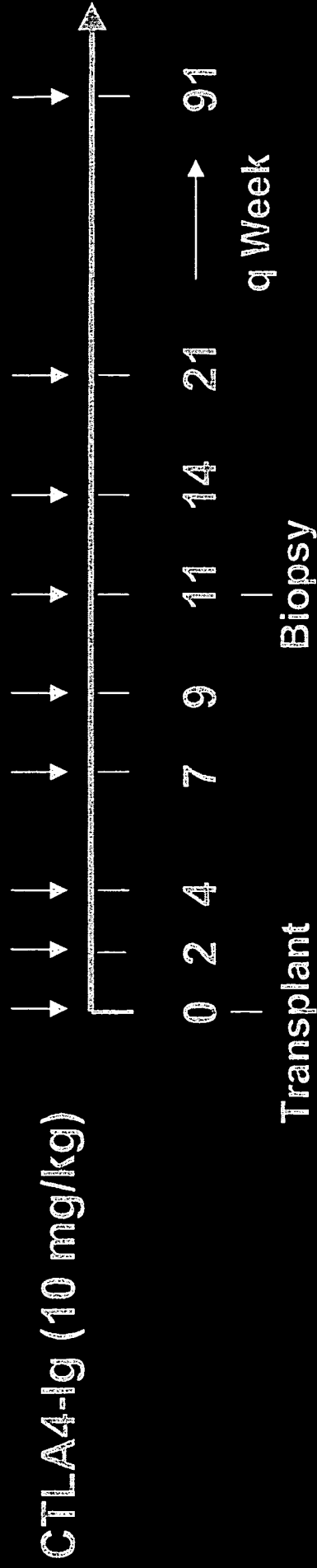
CTLA4-Ig (16 mg/kg) —



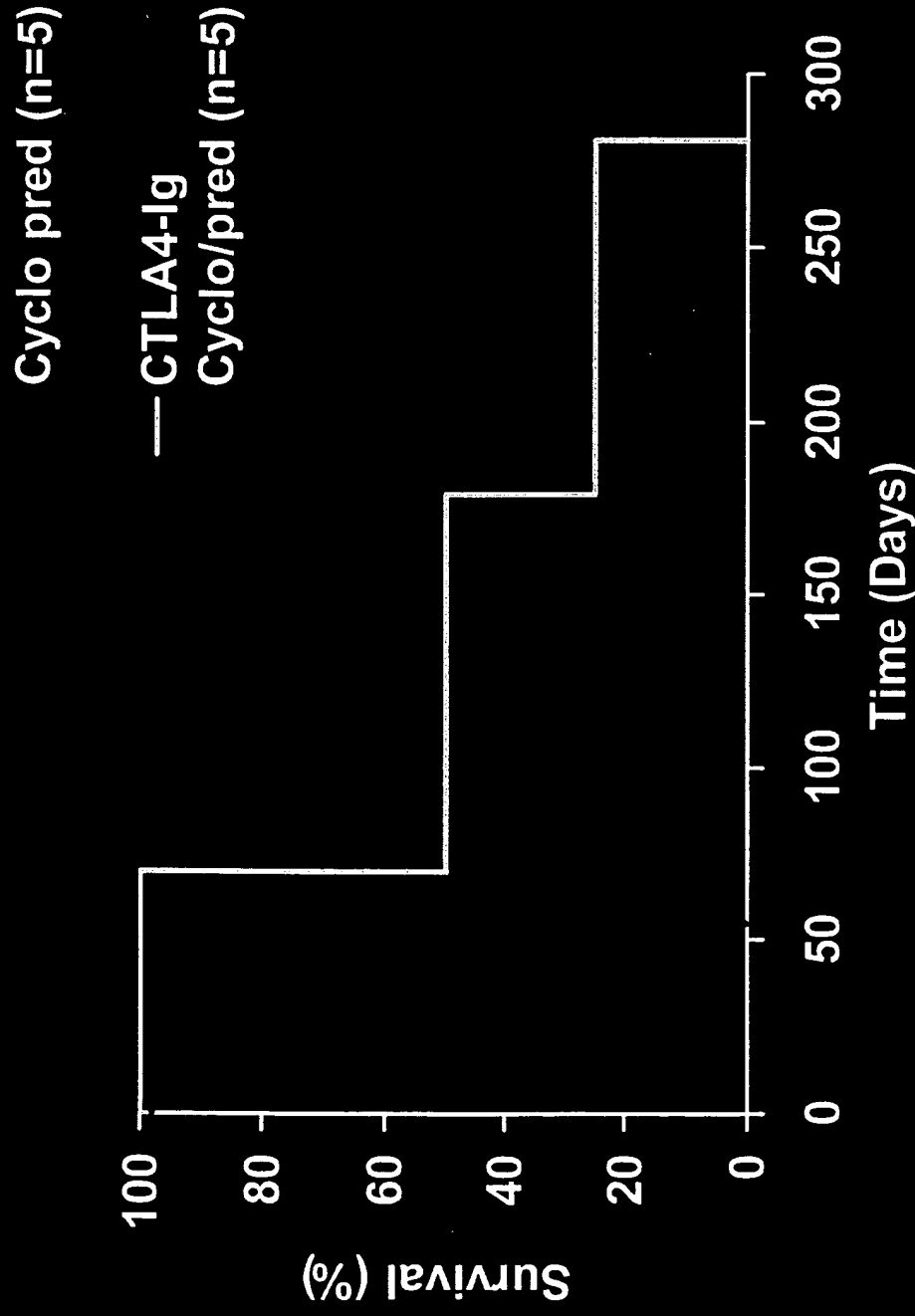
# CTLA4-Ig monotherapy minimally prolongs renal allograft survival



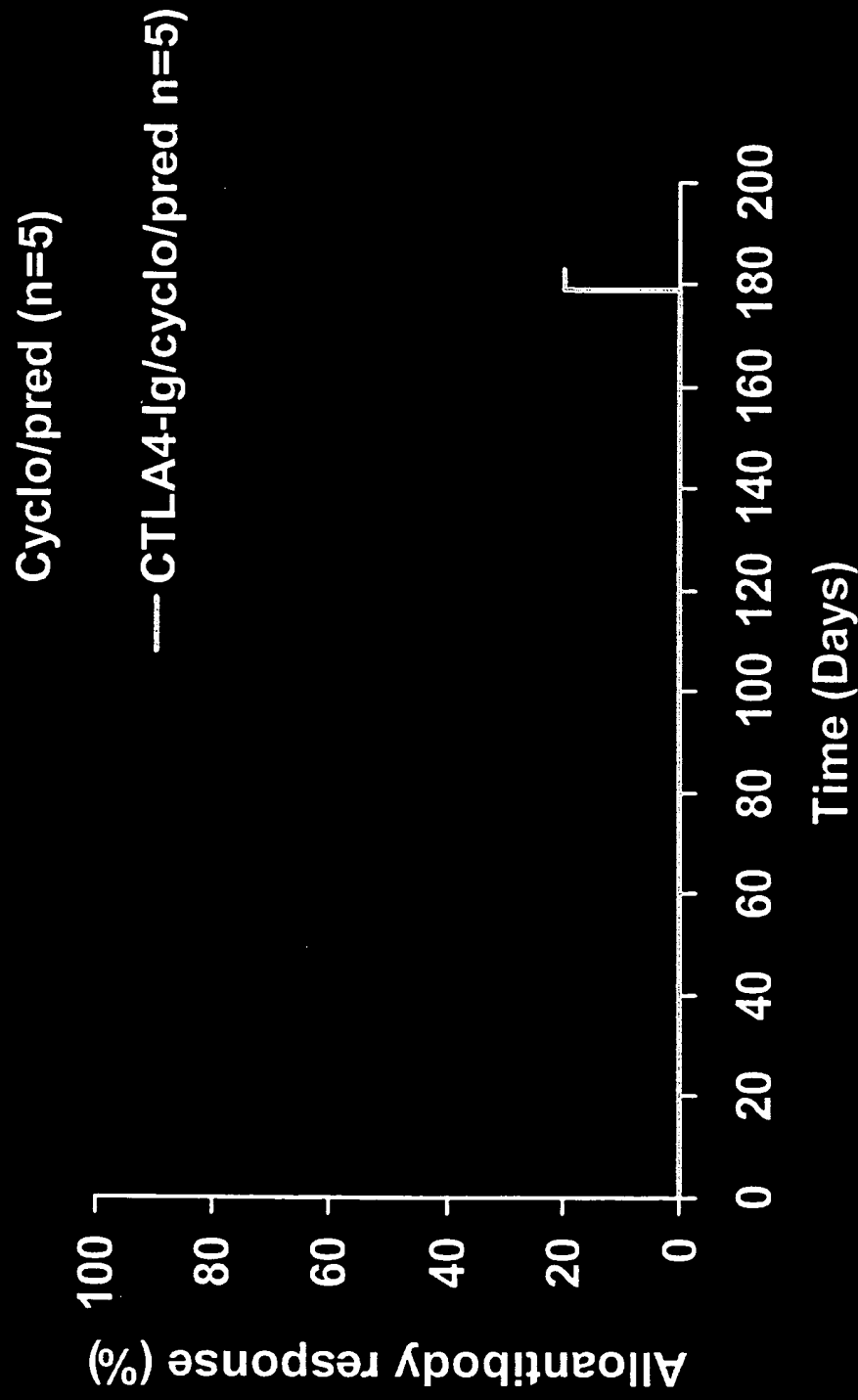
# CTLA4-Ig and conventional immunosuppression



# CTLA4-Ig prolongs allograft survival when given with conventional immunosuppression



# CTLA4-Ig inhibits anti-donor antibody responses



## Summary

- CTLA4-Ig is well-tolerated and immunosuppressive in non-human primates
- CTLA4-Ig inhibits anti-donor Ab responses
- immunosuppressive effects of CTLA4-Ig and cyclosporin are additive in the Rhesus renal allograft model